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The object of this project is to design and synthesize totally new, totally synthetic small molecules having specific enzyme activity. The ultimate goal is to design and synthesize a molecule with cholinesterase activity. The initial goal is to design and synthesize a molecule with chymotrypsin activity. Computer-assisted molecular graphics is being used to model chymotrypsin from the known X-ray structure and extract the amino acid residues responsible for catalytic activity and substrate binding. A synthetic framework of amphipathic helical peptides is being designed and will be synthesized to hold the essential amino acid residues in the conformation necessary for enzyme activity.

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The objective of the project is to model, design, and synthesize an enzyme structure that mimics the activity of acetylcholinesterase (AChE). While cloning techniques have produced knowledge of the primary amino-acid sequence of AChE, the three dimensional structure is not known.[5] Dissociative analysis of the native enzyme from *Torpedo californica* indicates the existence of two distinct molecular classes and at least six forms. The first class involves globular, hydrophobic structures that exist as dimers while the second involves elongated forms containing structural units attached to catalytic units by disulfide linkages. In addition, labeling studies implicate Ser200 as the residue responsible for hydrolysis of ester substrates.[4]

Our approach is to design structural subunits of parallel amphiphilic helices linked together covalently at the C-termini with catalytic regions placed near the N-termini. Initially, we will use the catalytic region from an enzyme with known crystal structure. Experience with this structure will indicate the modifications required to mimic the behavior of AChE.

The enzyme for the initial phase of the project is α-chymotrypsin since the crystal structure is known and the catalytic mechanism involves a "proton-transfer relay" system based on a reactive serine site similar to that inferred for AChE. Additionally, synthetic models of α-chymotrypsin built using cyclo-dextrins show catalytic activity over a limited pH range.[2] Using L-amino acid helices as the structural backbone to support the catalytic site should produce enzymes that are active under biologically functional conditions. We have initiated a modeling effort using the MENDYL (Tripos Associates, St. Louis, MO) and MIDAS (Univ. of Calf. San Francisco, CA) software running on the IRIS 3130 Workstation (Silicon Graphics Inc. Mountain View, CA) and have integrated the Brookhaven Protein Database (Brookhaven National Laboratory, Upton, NY) with the modeling software.

The overall properties of the model are summarized as follows. The catalytic and binding site environment in α-chymotrypsin is extracted from the Brookhaven protein coordinate database(file 5cha).[7] The catalytic residues are incorporated into helical peptide segments at the N-termini. The segments are placed in proximity to each other by fitting the catalytic site side chain atoms to the extracted Brookhaven structure. This yields segments whose relative orientation is determined by the catalytic site residues. These are subsequently aligned by fixing the catalytic site atoms and altering the appropriate side chain torsion angles to bring the helical cylinders into parallel alignment. The resulting structure provides a model for study of the interaction of the side chains and possible mechanisms of covalently linking the C-terminal ends.

The A side catalytic and binding site areas as extracted from the molecule are shown in stereo in Figure 1. There is a cleft that exposes the reactive serine(Ser195) to the substrate. The serine residue and binding site are located on one side of the cleft while the remaining residues of the "proton transfer relay" system (His57 and Asp102) are located on the other side. The binding site residues (Ser189-Ser195, Ser214-Cys220, Pro225-Tyr228) are shown highlighted in the figure. Collectively, they form a pocket for attachment of the substrate.

Figure 2 shows a stereo view of the catalytic site residues. The important catalytic relationships are shown by dashed lines. Throughout the design, the side chain atoms of these three residues are held fixed.

Figure 3 abstracts the geometry of Figure 2 to indicate the relative positions of the α carbon atoms. The three point geometry indicates that the serine residue is separated by 7.5A from aspartic acid and by about 8.6A from histidine with respect to the x-z plane. This implies that His and Asp should be separated by one or no α-helical positions (d=1.5 A for an α-helix), and Ser should be separated by five positions from Asp and either five or six positions from His. Thus, the helical segments will be offset by these amounts with respect to each other in the design. If the C-terminal residues are to lie in the same plane, the Asp and His chains will be five and six residues shorter then the Ser chain. Also of interest is the close proximity of the serine and histidine chains along the x direction in the figure. This restricts how the side chains built on these residues can be arranged without significant overlap.

Figure 4 shows the result of building helices on to the catalytic site residues prior to alignment. Only the backbone atoms of the helix chains are shown for clarity. The procedure for aligning the helices consists of altering the side chain torsional angles of the catalytic site residues until a parallel structure is achieved. There are five angles of interest (Hisχ1, Hisχ2, Aspχ1, Aspχ2, and Serχ1). To systematically search the angle combinations, the Asp angles and the Ser angle are varied to produce a set of combinations that align the Ser and Asp chains. The His angles then need only be scanned for each of the valid combinations in the previously defined set. This reduces the number of calculations significantly. The determination of whether two helices are parallel involves evaluating the cross product between equivalently defined vectors in each segment. When the cross product ($\mathbf{A}\times\mathbf{B}=|\mathbf{A}||\mathbf{B}|\sin\tau$) evaluates to zero, the vectors will be parallel and the helices will be aligned. From the set of allowed(parallel) conformations thus defined, the best will be selected interactively with respect to steric restrictions and the ability to maximize side chain interactions. We are currently working with the

software to obtain an efficient method of doing these calculations.

An example of one solution obtained interactively is shown in Figure 5. The top set of structures shows the helices viewed end on while the bottom set shows a side view orthogonal to the top set.

Once the desired aligned conformations are determined, side chains are assigned to maximize the probability of helix formation and the internal hydrophobic-hydrophobic side chain interactions. The optimal selection of residues is obtained through iterative calculation of the helix dipole moment and use of the secondary structure predictive methods of Chou and Fasman[1] and Garnier.[3] The dipole moment calculations are done using the Mendyl software and the predictive methods using the Microgenie software (Beckman Instruments Inc). Maximizing the interactions involves using dot surface intersections to evaluate the degree of interaction. The steps involved are outlined below.

    A. Determine an initial set of residues designe to maximize helix formation.

    B. Energy minimize the structure to resolve any bad contacts using the conjugate gradient minimizer in MENDYL.

    C. Define an interaction surface for each hydrophobic side chain atom based on the Van der Waals radii.

    D. Calculate the volumetric intersection of the defined surfaces.

    E. Identify areas that contribute small amounts to the overall interaction volume.

    F. Alter the residues within the hydrophobic set to increase the interaction volume.

    G. Recalculate the net dipole and secondary structure prediction parameters.

    H. Go to B. until the structure is satisfactory.

The work to date on this portion of the design has involved enhancing the software to do the area calculations and associating charges with the atoms according to the models of Sheridan for use in the dipole calculations.[6]

Additional consideration should be given to design of a suitable binding structure. Here there are two possibilities. First, one could use the clefts formed by

adjacent helical segments. Side chain residues in the
appropriate places could provide substrate-specific binding
"hooks". Another approach would use another helix in
proximity to the existing ones to mimic (for example) the
hydrophobic pocket of α-chymotrypsin. This method has the
advantage that it is independent of the structural chains.

The final portion of the design involves determining
appropriate covalent linkages for the C-terminal ends of the
helices. Preliminary analysis shows that the use of di-
amino acids at the C-terminal ends of the chains with or
without spacer residues should serve to hold the chains
together adequately. Control over the stereochemistry and
conformation is achieved using pseudo-peptide bonds between
the C-terminal residues or among the spacer residues if
required.

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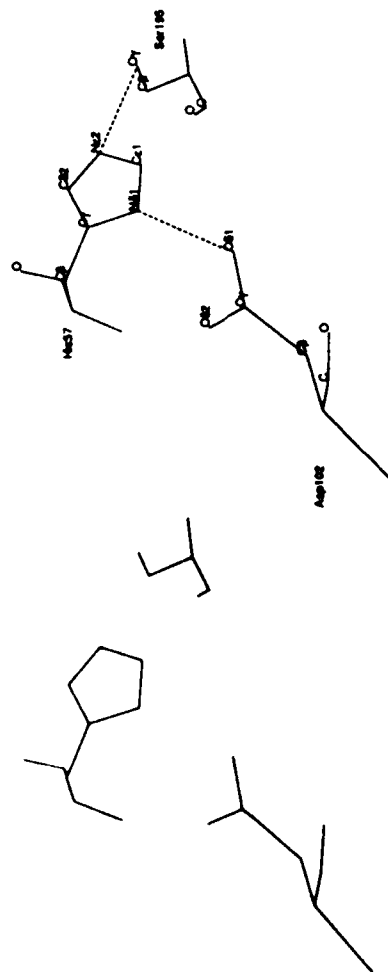α-Chymotrypsin Catalytic and Binding Site Environment
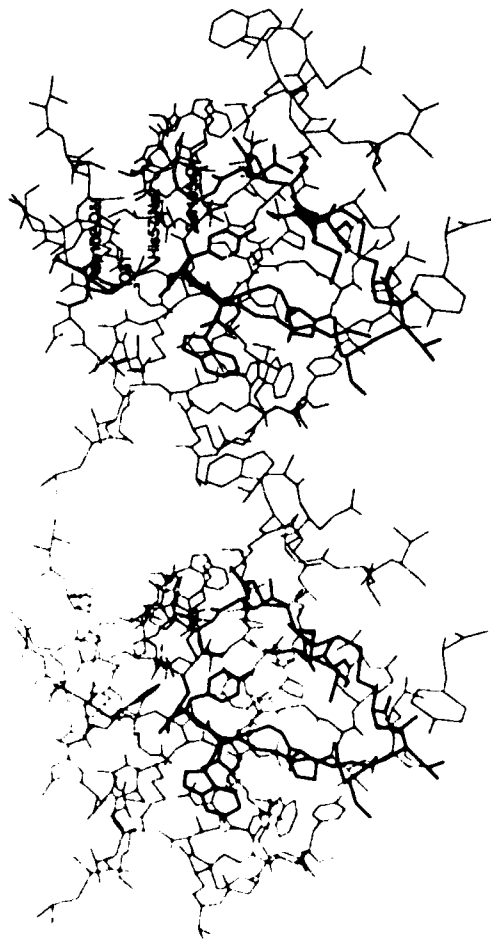
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Figure 1 Chymotrypsin catalytic and binding site environment. The binding pocket residues are highlighted to indicate the shape of the binding pocket. The Catalytic site residues are labeled at the proton transfer system atoms.

α-Chymotrypsin "Proton-Transfer Relay" System Residues

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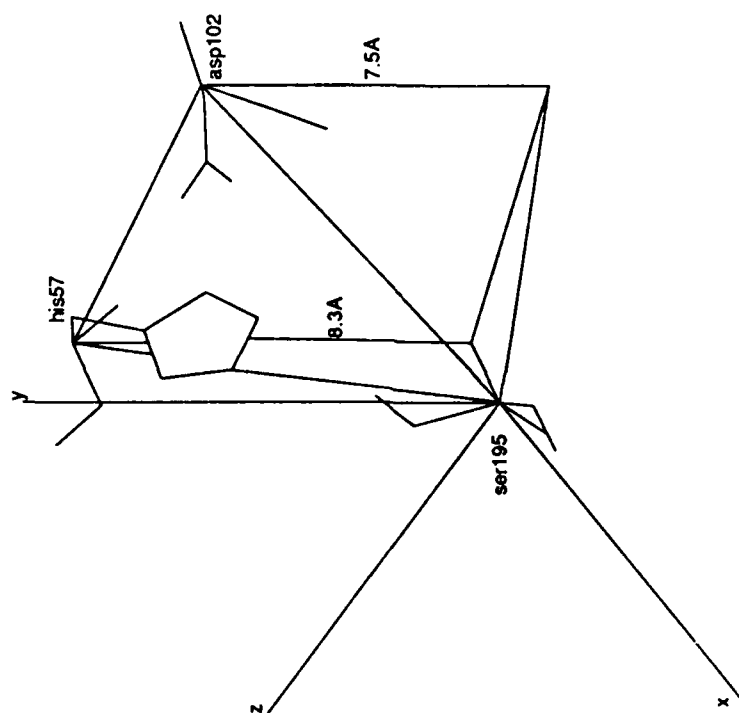
Figure 2: Chymotrypsin catalytic site residues as extracted from the Brookhaven Protein Database (5cha). The catalytic proton transfer mechanism is indicated by the dashed lines. Note the relative positions of the α-carbons and terminal oxygens.
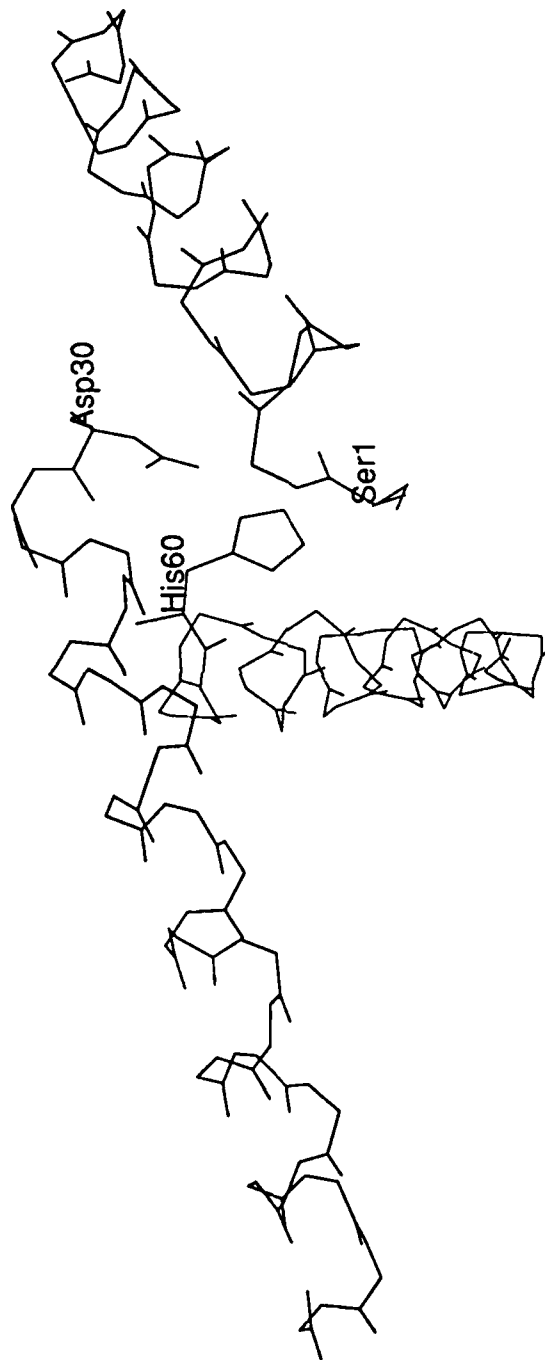
α-Chymotrypsin Catalytic Site Geometry

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Figure 3:Extracted Catalytic Site Geometry for α-chymotrypsin.

Unaligned Trihelizyme Framework

Asp30

His60

Ser1
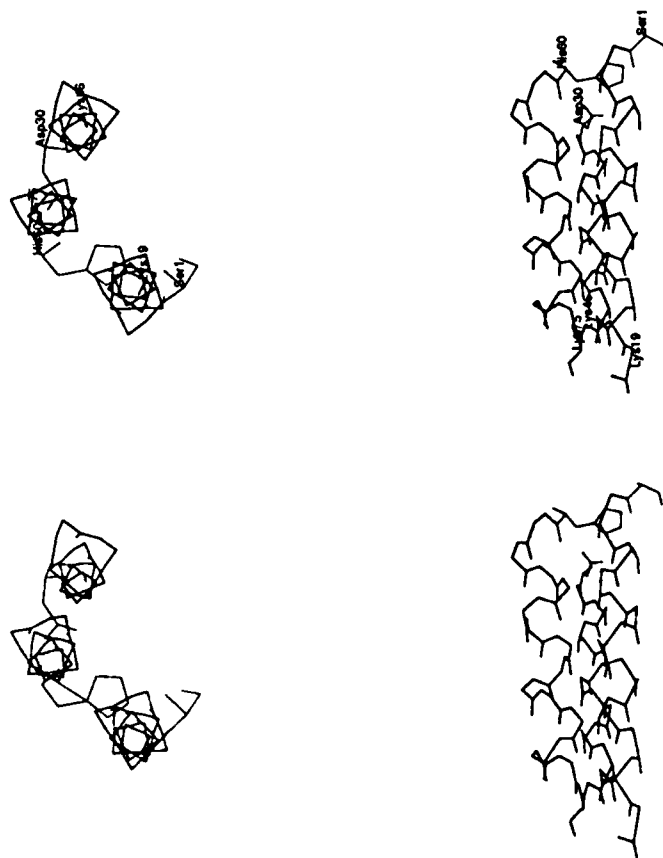
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Figure 4:Peptide helices built onto the catalytic site residues of α-chymotrypsin prior to alignment. The sidechains are not shown for clarity.

Parallel Aligned Trihelizyme Framework

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Figure 5:Two stereo views of a Trihelizyme structure based on the catalytic residues of α-chymotrypsin. The top set shows the structure looking end on while the bottom set shows the side view orthogonal to the top set.

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